Effects of microclimate and oviposition timing on prediapause larval survival of the Bay checkerspot butterfly, *Euphydryas editha bayensis* (Lepidoptera: Nymphalidae)

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Abstract. We tested empirically whether microclimate and relative timing of oviposition affected prediapause larval survival and development rates in the federally threatened Bay checkerspot butterfly, *Euphydryas editha bayensis* (Nymphalidae). Most mortality in Bay checkerspot butterflies occurs among prediapause larvae. Because phenology of the butterfly's larval hostplant, *Plantago erecta*, has been thought to drive prediapause larval survival patterns, we also tested whether *P. erecta* senescence and density over time varied among microclimatic zones. We found that microclimate had a significant effect on *P. erecta* phenology. Changes in density of edible *P. erecta* among microclimatic zones were out of phase temporally, but otherwise were similar. In the year of our study, neither microclimate nor oviposition date tended to affect prediapause larval survival, but both variables had significant effects on prediapause larval development rates. Because temperature and precipitation patterns in the butterfly's environment vary from year to year, whether microclimate and oviposition date significantly affect prediapause larval survival and development also may vary annually. At least in some years, however, senescence of *P. erecta* may not cause prediapause larval mortality. Our results support the hypothesis that topographic heterogeneity is critical to the long-term viability of the Bay checkerspot butterfly as well as other species that inhabit temporally variable environments.

Key Words: *Euphydryas editha bayensis*, invertebrates, conservation, microclimate, grasslands

Introduction

Spatial extent of suitable habitat is a fundamental consideration in conservation planning for viable populations of virtually all species. Certain landscape attributes that must be emphasized in conservation planning for invertebrates, however, differ from those that traditionally have received attention in conservation efforts targeting large vertebrates (Ehrlich and Murphy 1997). Habitat area is a primary concern for conservation of large vertebrates. These animals often require sizable protected zones in which population sizes can be maintained at or above a probabilistically safe

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baseline—for example, a 99% probability of remaining extant for 1000 years (Shaffer 1981, Boyce 1992). Not only geographic extent per se but also topographic heterogeneity of protected areas may be critical for the conservation of many invertebrates and small vertebrates, including the Bay checkerspot butterfly (*Euphydryas editha bayensis*) (Nymphalidae: Nymphalinae) (Ehrlich and Murphy 1987, Weiss *et al.* 1987, 1988, Launer and Murphy 1994). Spatial heterogeneity is important because invertebrate population dynamics frequently are density-independent and highly sensitive to climatic variability (Andrewartha and Birch 1954, Pollard and Yates 1993, DeVries *et al.* 1997, Crisp *et al.* 1998, Shaffer *et al.* 1998).

The Bay checkerspot butterfly, which inhabits patches of native serpentine soil-based grassland south of San Francisco, California, was listed in 1987 as threatened under the U.S. Endangered Species Act. Serpentine-based soils have a physical and chemical composition that limits the invasion of introduced Eurasian grasses, and thus can provide refugia for native vegetation (Kruckeberg 1954, 1984, Walker 1954, Thomas 1961, Turitzin 1981, Huenneke *et al.* 1990). The viability of these native grasslands and of the Bay checkerspot butterfly currently is jeopardized by suburban development (Murphy and Ehrlich 1980, Ehrlich and Murphy 1981, 1987). Conserving serpentine patches in the region is essential because the Bay checkerspot butterfly is structured as a "mainland-island" metapopulation in which local demographic units frequently go extinct and temporarily unoccupied habitat patches are recolonized (Ehrlich *et al.* 1975, 1980, Murphy and Ehrlich 1980, Ehrlich and Murphy 1981, 1987, Harrison *et al.* 1988).

Prediapause Bay checkerspot butterfly larvae suffer far greater mortality than any other life stage (Singer 1972, Ehrlich *et al.* 1975, 1980, Weiss *et al.* 1988, Cushman *et al.* 1994). Previous field studies estimated that survival of prediapause larvae rarely exceeds 10% annually (Singer 1972, Ehrlich *et al.* 1975, 1980, Singer and Ehrlich 1979, Dobkin *et al.* 1987, Weiss *et al.* 1988). Two interacting factors—microclimate and timing of oviposition during the growing season—are thought to affect rates of prediapause survival. Prediapause larval survival is believed to be highest among offspring of early-flying females that oviposit on cool north-facing slopes (Weiss *et al.* 1987, 1988, Murphy *et al.* 1990). On these slopes, the butterfly's larval hostplants [*Plantago erecta* (Plantaginaceae) and less commonly *Castilleja densiflora* or *C. exserta* (Scrophulariaceae)] remain edible until relatively late in the flight season (Weiss *et al.* 1987, 1988). Paradoxically, the females that fly earliest tend to be those that fed and pupated on warmer south-facing slopes, where hostplants senesce early and prediapause survival rates are thought to be lowest (Ehrlich *et al.* 1980, Weiss *et al.* 1988, Murphy *et al.* 1990). Eggs laid well into the flight season may be too late to produce larvae that survive on any slope (Weiss *et al.* 1988). For example, Cushman *et al.* (1994) estimated that just 1 week into the flight season, female reproductive success was less than 25% of that on the 1st day of the flight season. To date, estimates of prediapause larval survival over space and time have been based on measurements of hostplant senescence (Cushman *et al.* 1994) rather than measured

directly. The purpose of this study was to test empirically the influence of microclimate and relative timing of oviposition on prediapause larval survival. In addition to quantifying hostplant senescence and density over time in different microclimatic zones, we monitored the survival and development rates of prediapause Bay checkerspot larvae that resulted from eggs laid in different microclimatic zones on different dates during the flight season.

Study system

Euphydryas editha bayensis is univoltine. Adults fly for 3-5 weeks between late February and early May (Weiss *et al.* 1988). Females lay masses of 20-200 eggs near the base of larval hostplants (Singer 1972, Weiss *et al.* 1988). Newly-hatched larvae feed until they reach the 3rd or 4th instar and then enter an obligatory diapause that lasts through the dry season (approximately May-November) (Ehrlich 1965, Singer 1972). If hostplants senesce before larvae reach the middle of the 3rd instar, the larvae starve prior to or die during diapause (Singer 1972, Singer and Ehrlich 1979). When the rainy season begins, surviving larvae break diapause and feed on newly germinated *Plantago erecta* until February or early March (Singer and Ehrlich 1979, Weiss *et al.* 1988). Adults emerge following 10-20 days of pupation and generally live for 1-2 weeks (Ehrlich 1965, Murphy *et al.* 1983, Cushman *et al.* 1994).

Extreme weather events can have markedly deleterious effects on Bay checkerspot butterfly metapopulations (Singer and Ehrlich 1979, Ehrlich *et al.* 1980, Murphy and Ehrlich 1980, Murphy *et al.* 1990). When seasonal precipitation is average or slightly above average, and the rainy season is not prolonged, the geographic distribution of the butterfly tends to expand and population sizes often increase. When precipitation patterns are extreme (drought or deluge), however, or when the start of the flight season is delayed by cool and cloudy weather, the geographic distribution of the butterfly tends to shrink and its abundance tends to decline (Singer and Ehrlich 1979, Ehrlich *et al.* 1980, Dobkin *et al.* 1987, Weiss *et al.* 1987, Murphy *et al.* 1990).

Because variation in aspect and tilt affects solar exposure and retention of soil moisture, local topography within habitat patches mediates hostplant senescence and therefore plays a key role in enabling Bay checkerspot butterfly metapopulations to survive extreme weather events (Ehrlich and Murphy 1987, Weiss *et al.* 1987, 1988). For example, south-facing slopes receive more solar radiation on clear days, thus are warmer and drier than north-facing slopes. *Plantago erecta* on south-facing slopes often senesce 3-4 weeks prior to those on cooler north-facing slopes (Weiss *et al.* 1988). Because hostplants on relatively cool slopes remain edible long into the spring, those slopes are believed to serve as "core" habitat for the Bay checkerspot butterfly. The availability of even a few cool slopes within a habitat patch can prevent its butterfly population from being extirpated during a short or mild drought. The importance of warmer slopes to the persistence of Bay checkerspot butterfly populations should not be underestimated, however (Harrison *et al.* 1988, Weiss *et al.* 1988). Even very warm

slopes contribute to long-term viability of the Bay checkerspot butterfly by providing diverse early-season nectar, which can increase female fecundity and lifespan (Ehrlich and Murphy 1981, 1987, Murphy *et al.* 1983, Boggs 1997). Proximity of different microclimatic zones also is important because postdiapause larvae that disperse from cooler to warmer slopes may advance their adult emergence dates by a week or more, thus increasing their chances of reproductive success (Weiss *et al.* 1987, Cushman *et al.* 1994). In sum, survival and reproduction of the butterfly can occur under most macroclimatic conditions in a patch of habitat that includes a range of slope classes (Weiss *et al.* 1988).

Methods

Our experiments were conducted at Kirby Canyon, Santa Clara County, California, USA (37°11′ N, 121°40′ W) in spring 1993. This site includes approximately 1350 ha of serpentine soil-based grassland and is the butterfly's largest remaining habitat patch. The site is believed to serve as an important source of emigrants that recolonize adjacent habitat patches from which the butterfly has been extirpated (Harrison *et al.* 1988).

We selected 5 slopes as representatives of their microclimatic zones (Weiss *et al.* 1988, Cushman *et al.* 1994). Each was classified as very warm (south- and west-facing slopes, tilt >17°), warm (south- and west-facing slopes, tilt >11°), moderate (all aspects, tilt <11°), cool (north- and northeast-facing slopes, tilt >11°), and very cool (north- and northeast-facing slopes, tilt >17°). Replication of microclimatic zones was not tractable in terms of time and personnel requirements.

Plantago erecta phenology and density

To test the null hypothesis that *Plantago erecta* phenology does not vary among microclimatic zones, we monitored the phenology of 200 individual *P. erecta* through the Bay checkerspot butterfly flight season. Prior to the flight season, when virtually all *P. erecta* appeared edible (no visible senescence) and displayed only vegetative growth, we randomly selected 40 *P. erecta* in each of the 5 microclimatic zones. We monitored the phenology of each plant every 3-4 d over a period of 63 d, until all plants had senesced. Phenology was ranked on a qualitative scale from 1 to 5 (1 = strictly vegetative growth, 2 = partial flower, 3 = full flower, 4 = partial senescence, 5 = full senescence).

For each plant, we calculated the number of days between the start of the flight season and each phenological stage (from partial flower through full senescence). We conducted experimentwise comparisons of phenology (days from the start of the flight season to each phenological stage) with a nested analysis of variance using the General Linear Models Procedure (SAS 1990). Because microclimatic zones were subsampled rather than replicated, we used the interaction term as the error sums of squares; i.e., we calculated the *F*-value for each of the 4 analyses by dividing the microclimatic zone mean square by the mean square for individual *P. erecta* within all microclimatic zones. *P*-values reported for this and later analyses are for Type III sums of squares. When there was a significant microclimatic zone effect, we compared zones with Duncan's Multiple Range Tests. The significance level for these and later Duncan's Multiple Range Tests was set at alpha = 0.05.

We tested 2 hypotheses concerning the density of edible *Plantago erecta* during the Bay checkerspot butterfly flight season. First, we tested whether the density of edible

P. erecta varied among microclimatic zones at any given point in the flight season. Approximately once a week through the flight season, in each microclimatic zone, we measured the distance between 50 randomly selected, edible *P. erecta* and the nearest neighboring edible *P. erecta.* Plants were selected each week; we did not monitor the same plants over time. Measurements were made on 7 d over a 45 d period in all microclimatic zones. On Day 56, we only measured plants in the cool and very cool zones because we were unable to find 50 edible *P. erecta* in the other 3 microclimatic zones. We tested the effect of microclimatic zone on *P. erecta* density for each day on which measurements were made with analysis of variance using the General Linear Models Procedure (SAS 1990). When there was a significant microclimatic zone effect, we used least-squared differences to compare zones. The significance level for the latter tests was set at alpha = 0.05.

Second, we tested whether density patterns of edible *Plantago erecta* across time (rather than on individual days) varied among microclimatic zones. This hypothesis was tested with a General Linear Model *F*-test for detecting differences among regression lines (Neter *et al.* 1990).

Larval survival and development

To test the hypothesis that prediapause larval survival and rates of prediapause larval development did not vary among microclimatic zones and oviposition dates, we carried out the following protocol on each of 3 consecutive weeks during the flight season. Weeks 1, 2, and 3 approximately corresponded to days 7, 14, and 21 of the flight season. On the 1st day of each week, we captured at least 100 adult female Bay checkerspot butterflies at Kirby Canyon. We fed them a sugar solution *ad libidum* to encourage oviposition and then returned them to the field. In each microclimatic zone, we placed 20 females in cylindrical cages over edible *Plantago erecta* (one butterfly per cage). After several hours, we checked each caged site for presence or absence of an egg mass. Butterflies were removed from the cages and released in the area of capture.

We monitored the life stage of each group of offspring in the field every 2-3 d for 47 d, until all animals had either entered diapause or disappeared. Development usually was synchronous within each group. We scored the life stage of each group on a scale from 1-6 (1 = egg mass, 2-5 = 1st through 4th instars, 6 = diapause). Mortality of egg masses or 1st or 2nd instar larvae often can be observed directly. Prior to 3rd instar, disappearance also implies mortality (D.A. Boughton, unpublished manuscript). Many 3rd instar larvae disperse from the hostplant where they were deposited as eggs. These larvae are cryptic and extremely difficult to track as they move through the habitat. Dispersing 3rd instar larvae can molt and enter diapause after feeding briefly (D.A. Boughton, unpublished manuscript). They also, however, may starve or be depredated. Therefore, our hypotheses addressed survival to 3rd instar rather than to diapause. Because we were not able to monitor individual larvae, our measurements of survival and development corresponded to survival or development of at least 1 individual animal from each group.

We conducted Goldstein's x^*-tests (Goldstein 1964), controlling first for oviposition date and then for microclimatic zone, to test the hypothesis that survival to 3rd instar did not vary among microclimatic zones and oviposition dates. When there was a significant effect of microclimatic zone or oviposition date, we used Goldstein's x^*-tests to compare survival at different life stages (i.e., survival between egg and 1st instar, 1st and 2nd instar, and 2nd and 3rd instar).

To test the hypothesis that larval development rates did not vary among microcli-

Table 1. Effect of microclimatic zone on phenology of *Plantago erecta*. Values are mean ± σ days from the start of the Bay checkerspot butterfly flight season to each phenological stage. Black lines indicate means that are not significantly different (alpha = 0.05).

	Microclimatic zone				
Phenological stage	very warm	warm	moderate	cool	very cool
partial flower	13.6 ± 8.7	11.0 ± 6.7	11.3 ± 6.0	24.7 ± 5.5	28.9 ± 6.0
full flower	17.9 ± 8.6	15.4 ± 6.3	15.3 ± 5.5	28.7 ± 7.0	34.3 ± 6.6
partial senescence	26.0 ± 6.0	23.4 ± 4.2	25.2 ± 3.8	38.2 ± 5.8	43.8 ± 3.8
full senescence	34.4 ± 6.3	31.0 ± 6.0	33.2 ± 6.1	45.8 ± 4.0	49.3 ± 4.4

matic zones and oviposition dates, we calculated the number of days between oviposition and each larval instar for each group of offspring. We conducted experimentwise comparisons of the days to 1st and 2nd instar with a two-way analysis of variance using the General Linear Models Procedure (SAS 1990). Small sample sizes precluded comparison of later life stages. When there was a significant effect of microclimatic zone or oviposition date, we carried out among-zone and among-week comparisons with Duncan's Multiple Range Tests.

Results

Plantago erecta phenology and density

Numbers of days in each microclimatic zone from the start of the flight season to each *Plantago erecta* phenological stage are presented in Table 1. We rejected the hypothesis that *P. erecta* phenology does not vary among microclimatic zones. The experimentwise effect of microclimatic zone on *P. erecta* phenology was statistically significant ($P < 0.01$) for each phenological stage (partial flower: $F_{4,195} = 62.0$, full flower: $F_{4,195} = 63.5$, partial senescence: $F_{4,195} = 143.6$, full senescence: $F_{4,195} = 90.6$). *P. erecta* phenology was not distinct in each microclimatic zone, however (Table 1). Phenology of plants in the very warm, warm, and moderate microclimatic zones often was not significantly different (Table 1). Phenology of plants in the cool and very cool zones, by contrast, grouped neither with each other nor with plants in any of the warmer zones (Table 1).

Distances in each microclimatic zone from edible *P. erecta* to nearest neighboring edible individuals throughout the Bay checkerspot butterfly flight season are presented in Table 2. In each microclimatic zone, nearest neighbor distances across the flight season tended to decrease as new *P. erecta* germinated, then to increase as *P. erecta* senesced. The effect of microclimatic zone on nearest neighbor distances of edible *P. erecta* was statistically significant for each of the distinct points in time at which measurements were made, although the percentage of the variance in nearest neighbor distance

Table 2. Effect of microclimatic zone on density of apparently edible (no visible senescence) *Plantago erecta*. Values are mean ± σ nearest neighbor distances in mm. Degrees of freedom are 4,245 for days 1-45 and 2,98 for day 56. Black lines indicate means that are not significantly (alpha = 0.05) different. *** = $P \leq 0.0001$.

Day	Microclimatic zone						
	very warm	warm	moderate	cool	very cool	F	r^2
1	28.1±28.4	8.3±11.0	10.2±9.9	19.5±12.0	64.3±56.3	29.9***	0.328
8	17.6±20.5	8.1± 8.9	9.2±12.6	20.0±16.8	32.8±26.9	15.0***	0.196
14	16.9±55.6	3.4± 7.3	10.1±12.8	11.0±10.4	23.0±20.7	10.8***	0.150
21	22.2±34.4	7.2±10.5	19.6±26.9	16.1±20.2	29.9± 4.9	4.6***	0.070
28	62.9±84.0	41.4±32.6	40.7±45.5	25.2±20.4	28.5±33.5	4.7***	0.07
33	55.9±53.4	68.1±53.4	49.7±43.8	24.8±17.9	41.4±41.2	6.8***	0.100
45	129.4±94.2	114.9±87.2	135.2±82.7	38.3±36.1	43.1±42.9	21.4***	0.259
56				312.2±121.0	161.5±100.0	46.1***	0.320

explained by microclimatic zone often was small (Table 2). This result indicates that the relative timing of *P. erecta* germination and senescence varies among microclimatic zones. Significant differences ($P < 0.05$) in nearest neighbor distances among individual microclimatic zones are shown in Table 2. At the beginning of the flight season, edible *P. erecta* densities were greatest in the warm, moderate, and cool zones and lower in the very warm and very cool zones. From roughly the middle to the end of the flight season, the density of edible *P. erecta* was greatest in the cool and very cool zones.

Density patterns of edible *P. erecta* across the season as a whole (rather than on individual days) did not vary among microclimatic zones ($F_{12,22} = 0.69$, $F_{0.05, crit} = 2.23$, $P > 0.05$). In other words, density patterns among zones were out of phase temporally, but otherwise were similar.

Larval survival and development

Differences in *Plantago erecta* phenology are thought to be a key mechanism by which microclimate affects survival of prediapause Bay checkerspot butterfly larvae. We assumed *a priori* that the slopes on which we conducted our experiment had different microclimates (Weiss *et al.* 1988, Cushman *et al.* 1994). This led to the hypothesis that *P. erecta* senescence dates on each of the 5 experimental slopes would differ significantly. Our analysis of *P. erecta* phenology, however, rejected this hypothesis. Therefore, for analyses

Table 3. Number of groups of larvae with at least one representative surviving at each life stage.

	Microclimatic zone		
	warm group	cool	very cool
Week 1			
egg	34	16	5
1st instar	26	11	3
2nd instar	17	11	2
3rd instar	7	4	2
Week 2			
egg	24	16	4
1st instar	9	13	1
2nd instar	5	9	1
3rd instar	1	5	1
Week 3			
egg	28	8	8
1st instar	15	2	4
2nd instar	3	1	3
3rd instar	1	1	2

of larval survival and development, we grouped animals that had been deposited in the very warm, warm, and moderate microclimatic zones. We then tested whether (a) survival to 3rd instar and (b) development rates to 1st and 2nd instar differed significantly among 3 microclimatic zones (warm group, cool, and very cool) and among oviposition dates (weeks 1, 2, and 3). Sample sizes are presented in Table 3.

In most cases (8 of 9 tests), microclimatic zone did not have a statistically significant effect on survival to 3rd instar (Table 4). The single exception was that groups deposited in the middle of the flight season (week 2) had a greater probability of surviving to 3rd instar in the cool zone than in warm microclimatic zones. This largely was due to different probabilities of survival to 1st instar ($x^* = 2.725$, $P < 0.01$). Probabilities of survival from 1st to 2nd instar and from 2nd to 3rd instar were not significantly different between warm and cool zones on week 2 (1st-2nd: $x^* = 0.656$ ns, 2nd-3rd: $x^* = 1.288$ ns).

Likewise, only 1 of 9 tests showed a significant effect of oviposition date on survival to 3rd instar (Table 4). Groups deposited in warm zones on week 1 had a significantly higher probability of surviving to 3rd instar than did groups deposited in that zone on week 3. Survival from 1st to 2nd instar was higher in warm zones for those deposited on week 1 than on week 3 ($x^* = -2.800$, $P < 0.01$). Survival to 1st instar, and from 2nd to 3rd instar, however, was not significantly different between weeks 1 and 3 (egg-1st: $x^* = -1.896$ ns, 2nd-3rd: $x^* = -0.256$ ns).

Both microclimatic zone and oviposition date had a significant effect on rate of development from oviposition to 1st instar (microclimatic zone: $F_{2,79}$

Table 4. Goldstein's x^*-tests for survival to 3rd instar. * = $P \leq 0.05$ ($x^* \geq 1.960$), ** = $P \leq 0.01$ ($x^* \geq 2.576$), *** = $P \leq 0.001$ ($x^* \geq 3.291$).

Within week		Within microclimatic zone	
week 1	x^*	warm group	x^*
warm-cool	-0.351	weeks 1-2	1.786
warm-very cool	-0.962	weeks 1-3	1.989*
cool-very cool	-0.648	weeks 2-3	0.111
week 2		cool	
warm-cool	-2.350*	weeks 1-2	-0.393
warm-very cool	-1.498	weeks 1-3	0.711
cool-very cool	0.244	weeks 2-3	1.000
week 3		very cool	
warm-cool	-0.972	weeks 1-2	0.474
warm-very cool	-1.934	weeks 1-3	0.570
cool-very cool	-0.641	weeks 2-3	0.000

= 5.30, $P < 0.01$, oviposition date: $F_{2,79} = 44.80$, $P < 0.0001$) and from oviposition to 2nd instar (microclimatic zone: $F_{2,79} = 4.92$, $P = 0.01$, oviposition date: $F_{2,79} = 27.13$, $P < 0.0001$). The interaction of zone and date was not significant ($P = 0.19$) and therefore was removed from the model. Groups in warm zones developed more quickly than those in the cool zone (Table 5). Surprisingly, groups deposited in the very cool zone on week 1 also developed to 1st and 2nd instar more quickly than groups deposited in the cool zone on week 1 (Table 5). Relatively high densities of edible *P. erecta* (that is, limited senescence) may have accelerated the developmental rate of groups in the very cool zone. However, it is also possible that the accuracy of estimates of development rates in the very cool zone was affected by small sample sizes (Table 3). Within each microclimatic zone, mean rates of development were significantly different on weeks 1, 2, and 3. Groups that were deposited later in the flight season developed significantly more quickly (Table 5). As discussed below, the latter result was not independent of annual weather.

Discussion

It long has been assumed that interactions among topographic heterogeneity, hostplant senescence, and timing of oviposition mediate survival of prediapause Bay checkerspot butterfly larvae and, by extension, population sizes and geographic distribution of the butterfly (e.g., Singer 1972, Ehrlich *et al.* 1975, 1980, Ehrlich and Murphy 1987, Weiss *et al.* 1987, 1988, Cushman *et al.* 1994). In our experiment, microclimate had statistically significant effects on *Plantago erecta* phenology and density of edible individuals. In terms of *P. erecta* phenology, we found that microclimatic zones tended to group into three classes: warm, cool, and very cool. Similarly, by the middle of the flight season, when members of the earliest experimental cohort of offspring began to reach 1st instar and thus to feed, nearest neighbor

Table 5. Development times (mean ± σ) in d from oviposition to 1st and 2nd instar. Black lines indicate means that are not significantly (alpha = 0.05) different.

	Microclimatic zone		
	warm group	cool	very cool
1st instar			
week 1	15.9 ± 2.0	17.0 ± 2.3	15.7 ± 0.6
week 2	12.0 ± 2.1	14.8 ± 1.7	16
week 3	11.2 ± 1.4	11.3 ± 0.4	11.9 ± 1.4
2nd instar			
week 1	18.1 ± 1.8	19.3 ± 2.1	16.8 ± 1.1
week 2	13.7 ± 0.8	16.6 ± 1.5	17
week 3	11.9 ± 2.6	14	14.7 ± 1.2

distances of edible *P. erecta* often grouped among the very warm, warm, and moderate zones.

We found that microclimate had significant effects on rate of development to 1st and 2nd instar of Bay checkerspot butterflies. Oviposition date also had a significant effect on larval development rates to 1st and 2nd instar, although daily weather patterns represent a potential confounding factor. Because differences in annual weather patterns have complex ramifications for plant senescence and invertebrate population dynamics, whether oviposition date significantly affects larval development may vary annually.

Surprisingly, in the year that our study was conducted, neither microclimate nor oviposition date tended to affect survival to 3rd instar of the Bay checkerspot butterfly. Again, the effects of oviposition date on prediapause larval survival may depend upon annual fluctuations in temperature and precipitation. Caveats about temporal variability admittedly are frustrating; scientists and managers naturally would prefer clear-cut rather than equivocal experimental results. Yet variability and uncertainty are integral aspects of natural systems that inevitably must be addressed in developing conservation plans for species or ecosystems. Recent advances in conceptual development and implementation of adaptive management, which seeks to apply scientific principles to decision-making in the face of uncertainty, reflect growing recognition of the need to study and respond to shifting ecological conditions (McLain and Lee 1998, Slocombe 1998). Similarly, Gaston *et al.* (1998) argue that inability to conclusively accept or reject an ecological hypothesis should be viewed as an opportunity to focus on drivers and ramifications of variation rather than a deficiency of theory or method.

The absence of an effect of microclimate or oviposition date on larval survival in this experiment also may be in part an artifact of our study design. There is no tractable way to monitor individual prediapause larvae over many days if the larvae are allowed to disperse freely. Therefore, we quantified

survival at the group level rather than at the level of individual animals. If we had been able to track individuals, and most individuals deposited in the same egg mass starved before reaching 3rd instar or diapause, our survival estimates would be reduced dramatically. Conversely, our survival estimates might increase if many individuals that disappeared in fact survived to 3rd instar or to diapause. It is conceivable, although nearly impossible to quantify, that microclimatic zone and oviposition date have significant effects on the number of individuals per group that survive to diapause. We therefore agree with the inference of previous investigators that most reproductive females are likely to have some reproductive success, although the number of offspring per female that survive to diapause often decreases at later oviposition dates (Cushman *et al.* 1994).

Our results suggest that at least in some years, it is erroneous to assume that apparent senescence of *P. erecta* implies larval mortality (Ehrlich *et al.* 1975, 1980, Singer and Ehrlich 1979, Ehrlich and Murphy 1987, Cushman *et al.* 1994). For example, our data contradict the estimates of Cushman *et al.* (1994), which were based on hostplant senescence, that eggs laid after day 15 of the flight season (assuming a 28-day period of development from egg to diapause) or day 19 of the flight season (assuming a 24-day period of development) have no chance of reaching larval diapause. In our experiment, at least 1 individual from 4-31% of the egg masses laid on day 14 of the flight season (which developed to 4th instar in 25-28 days) survived to 3rd instar (the earliest stage at which larvae can enter diapause, Singer 1972). Similarly, at least 1 individual from 4-25% of the egg masses laid on day 21 of the flight season (which developed to 4th instar in 16-21 days) survived to 3rd instar. Again, our data cannot address the absolute number of individuals that survived, only the fraction of groups that had survivors. Moreover, the data of Cushman et al. were gathered in spring 1992, which was slightly warmer and drier than in 1993.

There are several possible explanations why we found that larvae survived after the majority of their hostplants had senesced. First, larvae may have developed on *P. erecta* that senesced later than most other *P. erecta* in the same microclimatic zone. Second, although *P. erecta* that have begun to senesce generally have been considered inedible (e.g., Cushman *et al.* 1994), prediapause Bay checkerspot butterfly larvae can eat *P. erecta* seeds that are green and developing even if the plant's flowers are dead (M.C. Singer, personal communication). Third, the mobility of 3rd instar larvae is considerable (mean = 17 mm in 10 min on warm sand; N. Mehdiabadi, Harrison, and C. Boggs, unpublished data), and these larvae may be able to seek out edible *P. erecta* even if those plants are few and far between. Fourth, it is probable that prediapause Bay checkerspot butterfly larvae are facultative cannibals (E. Fleishman, personal observation) that eat their siblings if edible hostplants are not available.

Previous work (e.g., Singer 1972, White 1974, Ehrlich *et al.* 1975, Weiss *et al.* 1988, Cushman *et al.* 1994) suggested that survival of prediapause Bay checkerspot butterflies occurs at the group level. In other words, if egg

masses each contained 100 eggs, then 99% mortality could imply that all individuals in one group survived and all individuals in 99 other groups starved. Our experiment suggests that survival instead may be spread widely among groups. Whether the former or latter scenario is more accurate has important ramifications for population dynamics and viability of the threatened Bay checkerspot butterfly. As distribution of survival among groups increases, so should the effective size (N_e) of the butterfly population, as well as its ability to withstand stochastic genetic events that can reduce probabilities of long-term population viability (Allendorf 1986, Frankham 1996, Rabinowitz *et al.* 1986).

Although hostplants senesce earlier in warm microclimatic zones than in cooler zones, distribution of offspring in warm as well as in cool zones likely increases the long-term viability of populations of the Bay checkerspot butterfly. For example, larvae that survive to diapause on warm slopes may have relatively high reproductive fitness as adults because they eclose earlier than individuals on cooler slopes in the subsequent year, when they have a good chance of finding mates and can lay eggs while hostplants are still young and edible (Weiss *et al.* 1988). Also, macroclimate in coastal California is notoriously unpredictable. Timing of *P. erecta* senescence relative to the Bay checkerspot butterfly flight season, and the magnitude of the difference in senescence timing among microclimatic zones, varies among years. Postdiapause larval densities in warmer microclimatic zones tend to increase in years following a relatively cool and wet flight season (e.g., Weiss *et al.* 1988).

Topographic heterogeneity likely is key to the persistence of numerous residents of native grasslands and other temporally variable environments. The need for topographic refugia may be especially pronounced among native annual plants, invertebrates, and other species with relatively short generation times or habitat requirements that vary thoroughout their life cycle.

Research on checkerspot butterflies (*Euphydryas*) in the western United States has been conducted virtually uninterrupted for the past 35 years. Biological studies of such duration are notable both for their rarity and for their ability to provide vital information for single- or multiple-species conservation planning (Ehrlich and Murphy 1987, Stohlgren *et al.* 1995, Heikkinen 1998). Nonetheless, our study emphasizes that it is critical to examine empirically our assumptions about long-term study systems.

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